GENIC ANALYSIS IN RICE

II. Inheritance of Resistance of Rice Seedlings to Blast Disease, *Piricularia Oryzae*[1]

by

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The blast disease caused by *Piricularia Oryzae* is the most important in the most rice growing countries. It seriously damages rice when nitrogen fertilizer is heavily applied. Therefore, breeding for blast disease resistance is urgently needed and a number of promising resistant varieties have been selected in various countries. However, the knowledges on the genetics of disease resistance are still limited.

In order to get more knowledge on the genetics of blast disease resistance in rice, the writers have conducted genetic experiment of leaf blast resistance in seedlings of rice. The preliminary results obtained through artificial inoculation are reported in this paper.

MATERIALS AND METHOD

Six resistant, six intermediate and seven susceptible varieties from Japan, U. S. A. and Taiwan were used as cross parents. 15 F_2 crosses together with their parental strains were planted in a specially designated green-house, in where artificial inoculation of blast fungus onto the seedlings was made.

Table 1. Strains used for genetic studies of blast disease resistance.

Strain No.	Variety name	Origin	Type of leaf blast resistance
P 123	Taichung No. 65	Taiwan (Ponlai)	Susceptible
P 124	Taichung No. 150	Taiwan (Ponlai)	Susceptible
P 130	Taichung No. 130	Taiwan (Ponlai)	Intermediate
P 138	Taichung No. 46	Taiwan (Ponlai)	Susceptible
P 141	Chianan No. 2	Taiwan (Ponlai)	Susceptible
P 163	Nung-yu g. No. 8	Taiwan (Glutinous)	Susceptible
P 167	Pai-kan-tao	Taiwan (Ponlai)	Resistant
T 208	Wu-liap--chin-yu	Taiwan (Native)	Resistant
T 215	Wu-liap	Taiwan (Native)	Resistant

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T 234	Wu-kao	Taiwan (Native)	Intermediate
J 302	Nakamura	Japan (Japonica)	Susceptible
M 503	Siau-nan-sien	China mainland	Intermediate
A 73	Nagao's gene marker	Japan	Susceptible
7101	Jodon's gene marker	U. S. A.	Intermediate
7114	Jodon's gene marker	U. S. A.	Resistant
7245	Jodon's gene marker	U. S. A.	Resistant
7247	Jodon's gene marker	U. S. A.	Resistant
9781	Jodon's gene marker	U. S. A.	Intermediate
9783	Jodon's gene marker	U. S. A.	Intermediate

The inoculum was composed of a number of isolates taken from infected leaves collected from various parts of Taiwan. Single conidia from single lesion was cultured in artificial media and the inoculum was applied in the form of a conidial suspension. The rice seedlings were grown in fungus free soil in iron flats (30 cm×30cm in size), placed in the green house. About 40–50 seedlings are planted in separated blocks in the same flat. Prior to the inoculation, 2% ammonium sulfate solution was top-dressed to the flats twice, at the rate of 25 cc per flat for each application. When the seedlings reach at the height of 15 cm, the conidial suspension was sprayed onto the leaves of seedlings. The inoculation was repeated 3–4 times so as to minimize the chance of escaping from infection. The flats were then placed in an inoculation chamber where the moisture (80–90%) and temperature (25–28°C) were controlled. A week after inoculation, the flats were moved out of the inoculation chamber into either a wire cage or in a green house, depending on the air temperature outside. Three to four days later, the inoculated seedlings were examined and records with regard to the type of disease lesions were taken according to the following six grades.

Grade	Degree of resistance	Lesion type	Description
O	HR	Immums	No visible sign of infection
A	R	Brown spot	Small brownish-dark brown lesions or spots.
B	MR	Brown margin	Brownish lesions with a distinct border, which would not increase in size.
C	MS	Grayish white center	Slowly expanding lesions restricted in size.
D	S	Large lesions with dark green center	Large lesions surrounded by brownish bands, expanding.
E	HS	Large lesions with grayish center	Water soacked appearance, often without well defined border, variable in size, lesions are expanded drastically.

Note: HR....Highly resistant
R....Resistant.
MR....Moderately resistant.
MS....Moderately susceptible.
S.....Susceptible.
HS....Highly susceptible

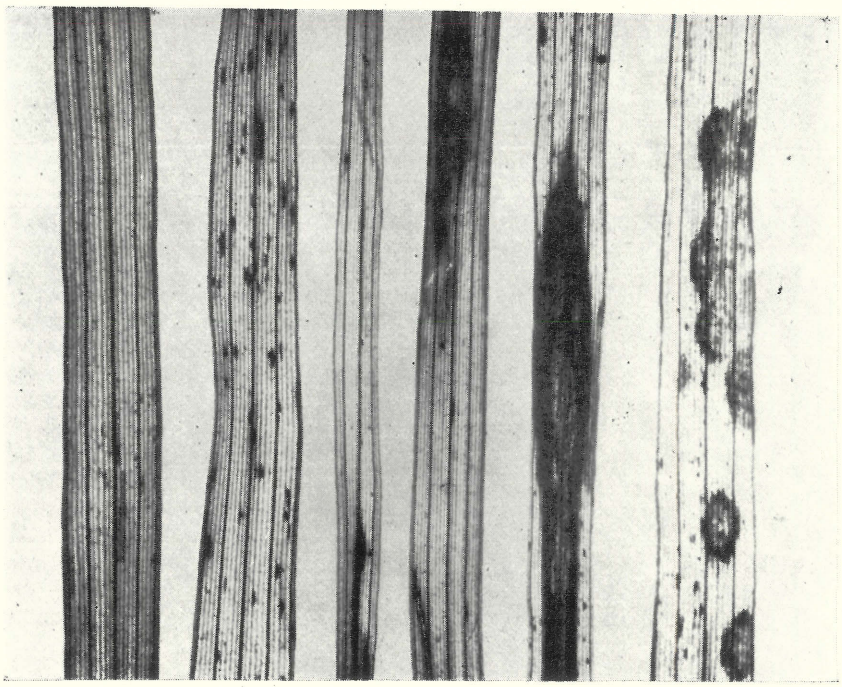

Fig. 1. Six infection types of leaf blast disease.

Figure 1 shows typical types of disease lesions. O, A, and D types were considered as resistant, while C, D and E types were considered as susceptible. The occurrence of blast infection of inoculated seedlings was satisfactory. It was however inevitable that, in some cases, classification was more or less arbitray, since the infection types in certain seedlings were hardly discernible.

EXPERIMENTAL RESULTS

Variation in seedling reaction to blast disease of parental strains and F_2s, obtained in accordance with the above mentioned classes are shown in Table 2.

As the table shows, crosses between resistant and susceptible strains, the F_2 segregated into 3 resistant and 1 susceptible plants in majority of the crosses, showing the resistance to be controlled by a single dominant gene. This result agrees with what Sasaki (1922) and Oka (1957) reported.

In the cross between a susceptible variety J302 (Nakamura, Japonica type) and a resistant variety T234 (Wukao, Indica type), the susceptible plant being smaller and the chi-square value did not show good fits to the expected 3:1 ratio. Since seed fertility in F_1 plant of this cross is 20%, gametic selection due to hybrid sterility may be one of the disturbing factors for such a large chi-square value. It is also possible that the excessive plants may also be due to "disease escaping". However, further studies are necessary for confirmation.

Table 2. Variations in seedling resistance to leaf blast disease in parental lines and F_2s in green-house experiment.

	Parents and crosses	Type of lesion O	A	B	C	D	E	Resistant (O+A+B)	Susceptible (C+D+E)	Total	Segregation ratio	X^2	P
1960	P123					115				115			
	P123×M503	15	8	12	18	10		35	28	63	9:7	0.013	0.8-0.9
	M503	1	3	8						12			
	P123×T208	59	9	43	20	11		111	31	142	3:1	0.760	0.3-0.5
	T208	54	46							100			
	P123×P167	72	28	19	14	13		119	27	146	3:1	3.297	0.05-0.1
	P167	64	17	10						91			
	P138					12	10			22			
	P138×7101	18	12	26	9	12		56	21	77	3:1	0.212	0.5-0.7
	P163	116	39	5	1	15	1			177			
	P163×7245	23	86	23	13	28		132	41	173	3:1	0.156	0.5-0.7
	7245	99	16	1	1					117			
	P163×9781	30	61	15	7	19		106	26	132	3:1	1.980	0.1-0.2
	9781	15	21	13	1					22			
	P163×9783	42	67	7	6	39		116	45	161	3:1	0.748	0.3-0.5
	P163×A73	31	50	8	1	32		89	33	122	3:1	0.273	0.5-0.7
	J302×T234	102	32	23	16	6		157	22	179	3:1	15.421	P > 0.01
	P141×P167	67	23	23	17	11		113	82	141	3:1	1.988	0.1-0.2
	P141					8				8			
1961	P123×P130	39 59		12 12		49 1		110	62	172	9:7	4.148	0.02-0.05
		98		24		50					9:3:4	3.266	0.1-0.2
	P130	22	37	5	4	30	2			100			
	P123×7114	34 77		10 16		61 2		121	79	200	9:7	1.468	0.2-0.3
		111		20		63					9:3:4	6.927	0.02-0.05
	7114	55	39	1	4	1		95	5	100			
	P124	45	20	3	8	24				100			
	P124×T215	34	22		5	31	2	56	38	94	9:7	0.421	0.5:0.7
	T215	14	35	15	2					66			
	P138×7247 (I)	11	17	7	12	2		35	14	49	3:1	0.333	0.5-0.7
	(II)	56	14	28	13	17	4	98	34	132	3:1	0.040	0.9-0.95
	7247 (I)	11	5	2	4					22			
	(II)	69	5	6	4	2				86			
	P123 (II)			1	3	64	9			77			
	P123×215 (I)	11	4	10	7	54	1	25	62	87	1:3	0.648	0.3-0.5
	(II)	20	3		3	58	6	23	67	90	1:3	0.015	0.8-0.9
	T215 (I)	14	35	15	2					66			
	(II)	70	26	2	2					100			

(I) Data of 1960. (II) Data of 1961.

The F_2s of the crosses, P123×M503, P123×P130, P123×7114 and P124×T215, 9 resistant and 7 susceptible plants were segregated. The chi-square value showed good fits to the expected ratio. When we classify the degree of resistance into three classes, resistant (O+A), intermediate (B+C) and susceptible (D+E) a 9:3:4 ratio was observed in P123×P130 and P123×7114. These facts indicate that at least two genes are

concerned with regard to leaf blast resistance. This assumption was confirmed by the second series of experiment conducted under the field condition.

DISCUSSION AND CONCLUSION

It will be concluded from the above mentioned results, that in the most crosses, the F_2s showed monogenic segregation, but some crosses showed a 9:7 ratio indicating that at least two genes are responsible for resistance to leaf blast. Sasaki (1922) reported that resistance to leaf blast is controlled by a single dominant gene. Nakamori (1926) and Hashioka (1950) concluded from their genetic studies that at least two dominant genes are responsible for resistance of blast disease.

A 9:7 ratio found in this study, whether represented a complementary effect of two genes or due to biased representation of a 9:3:3:1 ratio is a problem to be clarified. In view of 9:3:4 ratio found in some crosses, it is inferred that the resistance may be due to two dominant genes with supplementary effect. However, further studies are necessary to clarify this point.

The other thing to be considered might be whether or not there are physiological races with different reactions to varieties. Hashioka (1950) could not get a positive evidence for the presence of them. In Japan the existence of pathogenic races of blast fungus has been proved in the joint work done by various Agricultural Experimental Stations. Hokaido Agricultural Experimental Station (1957, 1958) made genetical studies on blast resistance in rice with race C-II, showing monogenic segregation in four out of six crosses. Whether other races will give the same pattern of segregation ratio to the same crosses is not known. In Taiwan Agriculturl Research Institute four pathogenic races have been identified. The writers are now on the way to study whether there is different reaction of different races to the segregation ratio of the above mentioned materials.

SUMMARY

Six resistant, six intermediate and seven susceptible varieties of rice from Taiwan, Japan and the U. S. A. were intercrossd, and were planted in a glass-house where humidity and temperature were controlled. Artificial inoculation of blast pathogen on the seedlings of rice was made and the infected leaves were exammined with regard to the type of disease lesions.

It was found that crosses between resistant and susceptible strains, in F_2, segregated into 3 resistant and 1 susceptible plants in majority of crosses, showing resistance to be controlled by a single dominant gene. In F_2s of some other crosses showed a 9:7 and 9:3:4 ratios indicating at least two genes are concerned with regard to blast resistance. The physiological races of pathogenes in relation to effect on the segregation ratio were discussed.

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稻之遺傳因子分析

第二報　稻苗對稻熱病抵抗性之遺傳

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摘　　要

就來自臺灣，日本及美國稻品種20種互交，並將其 F_2 及雙親，同時種在能調節溫度及濕度之玻璃室內。二星期後將經人工培養的稻熱病菌（*Piricularia Oryzae*）接種於稻苗上，病發後調查病葉的病斑型。

大部分的抗病×感病之雜交組合，在 F_2 分離爲 3 抗病 1 感病之比率，表示抗病受一對顯性遺傳因子支配。其他雜交組合則表示 9：7 及 9：3：4 之分離比，表示抗葉稻熱病至少受兩對顯性遺傳因子支配。稻熱病菌生理型對分離比之影響，亦曾加以討論。